THE ISOLATION OF MYCOPLASMA SYNOVIAE FROM CHICKENS WITH INFECTIOUS SYNOVITIS AND AIR-SACculITIS IN THE REPUBLIC OF SOUTH AFRICA

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ABSTRACT


Mycoplasma synoviae was isolated from the trachea of chickens showing either typical infectious synovitis lesions or air-sacculitis. M. synoviae was identified by means of the direct plate fluorescent antibody technique and its growth-dependence on nicotine-adenine dinucleotide.

This is the first documented report on the isolation and identification of M. synoviae in the Republic of South Africa.

Résumé

ISOLEMENT DE MYCOPLASMA SYNOVIAE À PARTIR DE POUSSINS ATTEINTS DE LA SYNOVITE INFECTIEUSE ET DE L’AEROCYSTITE EN REPUBLIQUE SUD-AFRICAINE

L’auteur a pu isoler Mycoplasma synoviae de la trachee de poussins avec manifestations cliniques soit de la synovite infectieuse soit de l’aerocystite. M. synoviae a pu être identifiée par la technique d’immuno-fluorescence directe sur plaque et en raison de sa dépendance du dinucleotide nicotinamide-adenine dans sa croissance.

Ceci est la première fois que l’on a rapporté l’isolement et l’identification de M. synoviae dans la République Sud-africaine.

INTRODUCTION

Yoder (1972) classified 19 serotypes (A-S) of Mycoplasma isolated from poultry. M. gallisepticum, M. gallinarum, M. meleagridis and M. synoviae are designated A, B, H and S respectively, while the other serotypes (C-R) have not been named.

M. gallisepticum, M. meleagridis and M. synoviae are considered to be pathogenic for certain avian hosts under various conditions, but the status of pathogenicity of the other serotypes is less clear (Yoder, 1972). M. synoviae is the cause of infectious synovitis in both chickens and turkeys (Olson, 1972) and of air-sacculitis (Kleven, King & Anderson, 1972; Vardaman, Reece & Deaton, 1973; Vardaman, Deaton & Reece, 1974; Gilchrist & Couttw, 1974; Gillespie, 1974).

In 1968, Du Preez (unpublished observation) isolated an organism suspected of being M. synoviae from the hock joints and footpads of broilers showing typical lesions of infectious synovitis. The isolation was done in embryonated eggs, and typical synovitis lesions could be reproduced in susceptible chickens infected with embryonic material. This isolate was not identified, however.

For the isolation of Mycoplasma, both solid and fluid media are normally used, the more common being C-agar and C-biphasic medium (Chalquest, 1962), as modified by Olson, Kerr & Campbell (1963), Mycoplasma agar and broth (Bradbure & Howell, 1974) PPL0 agar and broth (Timms, 1967) and medium FM -4 (Frey, Hanson & Anderson, 1968) as modified by Vardaman & Yoder (1969).

Frey et al. (1968) showed that nicotine-adenine dinucleotide (NAD) is essential for the growth of M. synoviae and distinguishes it from other avian Mycoplasma species. Olson & Meadows (1972) gave optimum and minimum concentrations of NAD: viz. for isolation—(0.01 and 0.00125%), for antigen production—(0.00125 to 0.01% and 0.000625%), and for maintenance—(0.00125 and 0.000312%).

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Olson & Kerr (1969) found that the use of embryonated eggs and C-medium was equally reliable for the isolation of M. synoviae, but that C-medium was better for the isolation of M. gallisepticum.

Olson & Kerr (1967) were able to isolate M. synoviae and M. gallisepticum from the footpad, hock joints, liver, spleen, trachea and blood of chickens. Bradbury & Howell (1974) isolated M. synoviae from the trachea, lung, airsacs, kidney, liver, heart, spleen and bursa of Fabricius of chickens hatched from infected eggs.

The identification of mycoplasmas is done by means of the fermentation of carbohydrates, complement fixation tests (Frey & Hanson, 1969), growth inhibition (Fabricant, 1960; Dierks, Newman & Pomeroy, 1967), polyacrylamide electrophoresis (Razin, 1968) and agar gel diffusion (Too, 1967). The staining of colony imprints on cover slips with fluorescent antibodies was used by Corstvet & Sadler (1964) while Del Giudice, Robillard & Carski (1967) stained Mycoplasma colonies on agar with fluorescent antibodies, using incident light for illumination.

The purpose of this article is to report the isolation and identification of field strains of M. synoviae from cases of synovitis and arthritis as well as cases of air-sacculitis in chickens, by the direct fluorescent antibody (FA) technique.

MATERIALS AND METHODS

Culture media and isolation procedure

Use was made of C-biphasic medium (Chalquest, 1962), modified by Olson et al. (1963), and further adapted by Du Preez (unpublished observations, 1973) by the omission of phenol red from the agar medium and the addition immediately before use of NAD* to the broth of the biphasic medium to a concentration of approximately 0.01%, C-agar plates with and without NAD at a concentration of 0.01% were also prepared.

* B.D.H. Chemicals Ltd., Poole, England
Routine cultures on C-agar plates and in C-biphase medium were made from tracheal swabs, airsacs and/or joints in cases where a Mycoplasma infection was suspected. The plates were incubated in humidified candle jars for a period of 14–28 days.

**Mycoplasma strains**

The avian Mycoplasma serotypes used in this study are given below:

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Isolate</th>
<th>Origin</th>
<th>Culture received from</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>801</td>
<td>Turkey airsac</td>
<td>Yoder</td>
</tr>
<tr>
<td>B</td>
<td>1504</td>
<td>Chicken trachea</td>
<td>Yoder</td>
</tr>
<tr>
<td>C</td>
<td>859</td>
<td>Chicken trachea</td>
<td>Yoder</td>
</tr>
<tr>
<td>D</td>
<td>8</td>
<td>Chicken trachea</td>
<td>Yoder</td>
</tr>
<tr>
<td>E</td>
<td>860 (DPR-2)</td>
<td>Chicken trachea</td>
<td>Yoder</td>
</tr>
<tr>
<td>F</td>
<td>1197 (BA)</td>
<td>Turkey trachea</td>
<td>Yoder</td>
</tr>
<tr>
<td>G</td>
<td>695</td>
<td>Turkey trachea</td>
<td>Yoder</td>
</tr>
<tr>
<td>H</td>
<td>866 (N)</td>
<td>Turkey airsac</td>
<td>Frey</td>
</tr>
<tr>
<td>I</td>
<td>694</td>
<td>Pigeon turbinate</td>
<td>Yoder</td>
</tr>
<tr>
<td>J</td>
<td>1853</td>
<td>Turkey</td>
<td>Olson</td>
</tr>
</tbody>
</table>

The serotypes listed above were again cloned and then used for production of hyperimmune sera (Du Preez, unpublished data, 1973). Conjugation with fluorescein was done by A. P. Schutte of this institute according to a method previously described (Schutte, 1969).

The staining method of Del Giudice et al. (1967) was modified by Schutte (personal communication, 1975) as follows: 10 blocks of the C-agar plate bearing typical Mycoplasma colonies with a surface area of approximately 0.5 cm² were cut out and each block was stained with a different serotype of fluorescein-conjugated rabbit antiserum. After staining, a thin horizontal slice of each block bearing the colonies was removed with a sharp blade and placed on a microscope slide. This was covered with a drop of FA mounting fluid® and a coverslip, and, after the air bubbles had been removed, it was examined with a Leitz universal microscope.

When pure M. synoviae cultures were identified by the FA technique they were cloned and then subcultured on C-agar plates with and without NAD.

The methods described here were applied to 23 trachea specimens, 9 from suspected cases of infectious synovitis, and 14 from cases with airsacculitis.

**RESULTS**

**Isolation from 9 synovitis cases**

*M. synoviae* was isolated from the tracheas of cases showing typical synovitis as described by Olson (1972). In every case the trachea was filled with creamy to caseous pus. Pure cultures of *M. synoviae* were obtained from 7 cases and 1 case revealed *M. synoviae* together with other serotypes (see Table 1).

**TABLE 1** Serological classification of the mycoplasmae from 9 typical infectious synovitis cases by the FA technique. All the isolates were from tracheal swabs

<table>
<thead>
<tr>
<th>No. of specimens</th>
<th>Serotypes isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

* Difco Laboratories, Detroit, Michigan, U.S.A. 48201

**Isolation from 14 cases with airsacculitis**

These cases had no history of synovitis but a very high incidence of airsacculitis. The tracheas of three of these cases revealed pure *M. synoviae* infection, and those of 9 cases pure *M. gallisepticum*. In 2 cases, either *M. synoviae* or *M. gallisepticum* was found together with other serotypes. (See Table 2).

**TABLE 2** Serological classification of the mycoplasmae from 14 cases with airsacculitis by the FA technique. All the isolates were from tracheal swabs

<table>
<thead>
<tr>
<th>No. of specimens</th>
<th>Serotypes isolated</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
</tr>
<tr>
<td>1</td>
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</table>

After 19 days incubation *M. synoviae* was isolated from the turkey trachea of 1 case by the FA technique. In all the other cases where *M. synoviae* was isolated, growth was first observed in the biphase medium after 3–7 days as indicated by a drop in pH. Typical *Mycoplasma* colonies developed after 3–5 days subsequent to the biphase medium being plated on C-agar.

Pure cloned *M. synoviae* cultures, identified by the FA technique, did not grow on C-agar plates without NAD.

**DISCUSSION**

This publication is the first report of the isolation and identification of *M. synoviae* in the Republic of South Africa. The isolation of *M. synoviae* from the trachea of cases of airsacculitis supports the conclusions of Kleven et al. (1972), Vardaman et al. (1973, 1974), Gilchrist & Cottew (1974), and Gillespie (1974), that this organism plays a role in respiratory disease of chickens.

The poor isolation results obtained when the swabs were streaked directly on C-agar might be attributed to the fact that the plates initially were kept for only 14 days, Bradbury & Houston (1974) suggested that plates for *M. synoviae* be kept for 28 days. In 1 instance where this was done, a few atypical colonies as described by them were observed on the 19th day. Contrary to their findings, no atypical colonies were found only in samples taken from the kidney, heart, spleen and bursa of Fabricius, these colonies originated from a sample taken from the trachea.

Du Preez's isolation in 1968 (unpublished observations) of a *Mycoplasma* that could reproduce synovitis in chickens coincided with the first occurrence of clinical infectious synovitis of nonbacterial origin in the Republic of South Africa. He subsequently established the FA technique in this laboratory for identification of 10 avian *Mycoplasma* serotypes. This proved to be a very practical method of identifying isolates of mycoplasmas, especially when several serotypes occurred in the same chicken. In fact, it was possible to identify 4 serotypes in a single specimen.

A combination of solid and biphase growth media facilitated the isolation of field mycoplasmae.
FIG. 1 *Mycoplasma* colonies stained with fluorescent antibodies. (a) A single colony of serotype D. (b) Serotype S colonies. The edge of the agar slice can be seen in the lower right-hand corner. (×128)
ACKNOWLEDGEMENTS

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REFERENCES


